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Analysis of Heavy Metals in Muscle Tissues of Tilapia Zilli of Gwale Pond, Kano State, Nigeria

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Abstract- Heavy metals such as nickel, copper, zinc and lead, generally are toxic substances that pose a great risk to living organisms in our environment due to their inevitable existence. The aim of this investigation is to investigate and assay the presence of such metals in muscle tissues of tilapia zilli of Gwale Pond in Gwale Local Government Area of Kano State, Nigeria. Muscle tissues of some fresh tilapia zilli were dissected, prepared and assayed by Alpha A4 atomic absorption spectrophotometer. The ranges of working standard concentrations were 0.001 ppm to 1 ppm .Study findings showed that the mean concentrations of nickel, copper, zinc and lead were 0.0120±0.002 ppm, 0.0637± 0.0035 ppm, 0.0097±0.0006 ppm and 0.0075±0.0005 ppm Respectively. The 95% confidence intervals for nickel, copper, zinc and lead were calculated to be 0.0120 ± 0.005, 0.0035 ±0.009, 0.0006 ±0.001 and 0.0005 ±0.001 respectively. The method was validated using triplicate analysis, recovery experiment and statistical analysis

Index Terms- Heavy metals, muscle tissue, tilapia zilli, gwale pond, atomic absorption spectrophotometer.

I. Introduction

Fish is said to be defined as a vertebrate adapted for a purely aquatic life propelling and balancing itself by means of fins and obtaining oxygen from the water for breathing purposes by means of gills [1]. The generic name of a group of cichlids endemic to Africa is Tilapia. The group consists of three aqua culturally important genera, Oreochromis, Sarotherodon and Tilapia zilli [2]. A wide variety of natural food organisms such as aquatic invertebrates, larval fish, plankton, some aquatic macrophtes, detritus and decomposing organic matter with heavy supplemental feeding are ingested by Tilapia [2]. Like other animals require common components of foods such as proteins, carbohydrates, fats, vitamins; minerals and water , Fishes also required such nutrients to enable them live comfortable life [3]. The most important food nutrient of interest is the mineral content of fish with respect to this research. There have been some difficulties in accessing the dietary requirements of minerals and other trace elements in fishes because of their ability to absorb elements directly from the water [4].

Heavy metals refer to any metallic chemical element that has relatively high atomic weight and toxic at low concentration. Air, soil, water, sediments and biota including aquatic organisms are reported to have been contaminated by heavy metals [4]. [4] found that the concentration of toxic heavy metals (Ni, Cu, Zn & Pb) in fish is affected by many biological factors, such as species, sex, age, feeding type), and environmental factors, such as the season of the year, pH value of water, temperature, dissolved oxygen and salinity. The elements enter the aquatic environment through weathering of the earth crust, human activities as well as industrial effluents [5]. Sources of heavy metals to the environment according to [6]. are mainly direct deposition from waste water from mining activities, domestic and industrial processes. For several decades, the increased effluents output has led to serious environmental pollution [7]. some prevailing conditions, heavy metals may accumulate to a toxic level [8] and suddenly result to ecological damage [5]. Nickel belongs to transition metals and has high metallic conductivity and ductility. It is paramagnetic, chiefly valuable for the alloys it forms, especially many super alloys particularly stainless steel. Copper is a chemical element with atomic number of 29. It is ductile with perfect electrical conductivity. Copper is required in the formation of elastin as well as collagen-making. It can also be used as an anti-germ surface that can add to the anti-bacterial and antimicrobial features of buildings such as hospitals [9]. Zinc is a trace element that is essential for human health. Lead is a chemical element with atomic number of 82. It is soft and malleable. It is used in building constructions, lead acid batteries, bullets and is part of solder, pewter, and fusible alloys.

Much work has been done with respect to the determination and analysis of heavy metals in varieties of fish species using spectrophotometers. It is crystal clear that researchers used different methods of anchoring fishes. Some uses nets where as some uses traps [10] and so on. Usually polyethylene bags are used for collecting anchored fishes to avoid sunlight interference. Storage temperatures differ from one region to another and from fresh waters, seas and marine fishes. Many researchers prefer ashing to wet digestion as ashing is more common, direct, easier and cheaper with automated electric furnace. The preference for instrumentation during the analysis depends on the availability and expertise of the researchers and the operators of the instruments since spectrophometer is a composite term which include ;flame atomic absorption, flame atomic emission, atomic absorption spectrometry , inductively coupled plasma etc. [10] determined seven heavy metals (Hg, Pb, Cd, Fe, Ni, Cu and Zn) in three fish species from coastal waters of Santa Cruz de Tenerife (Canary Islands) using atomic absorption spectrophometers. [11] used Scientific 210 VGP Atomic Absorption Spectrophotometer, to determine the concentrations of chromium, zinc and lead in muscle tissue of tilapia fish species from Dala and Gwale ponds in in Kano metropolis, Nigeria. A micro wave digestion was used by [12] during their study on Determination of Some Metal Levels in Muscle Tissue of Nine Fish Species from Beyşehir Lake, Turkey. An Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) was used by [13]. They all generated a remarkable result. A review of literature shows that both biological (species and feeding type) and the environmental factors affect the extent of contamination of fish muscle tissue with nickel, copper, zinc and lead. As a result, this study aimed at determining the level of heavy metals (Hg, Pb, Cd) in muscle tissues of three tilapia fish species anchored in Gwale Pond of Kano State, Nigeria and to evaluate their safety based on Food and Agricultural Organization [14] standard. As reported by [10] and [11], atomic absorption spectrophotometer was also used in this work.

II. STUDY AREA

Gwale is a Local Government Area in Kano State, Nigeria within Greater Kano city. Its headquarters are in the suburb of Gwale. It has an area of 18 km² and a population of 362,059 at the 2006 census. Its coordinates are 11°58′N8°30′E. Gwale Pond is located near its grave yard which is very close to Bayero University Kano old campus road. The main source of water for this pond is rain water. The pond also receives wastes and runoff water from nearby soils and waste deposits from human activities during rain fall.

III. EXPERIMENTAL

Sample Collection and Preparation

Three pieces of tilapia fish (tilapia zilli) were collected from Gwale pond of Gwale local government area of Kano State, Nigeria. The fishes were dissected and the muscle tissues extracted and dried at 110^{o}C in an oven. After drying, the samples were made into fine powders. Alpha A4 atomic absorption spectrometry model was used for the measurement.

Reagents

The following reagents were used; pure compounds of nickel chloride ($NiCl_2$), hydrated copper nitrate ($Cu\ (NO_3)_2.3H_2O$, hydrated zinc nitrate ($Zn\ (NO_3)_2.\ 6H_2O$, lead nitrate ($Pb\ (NO_3)_2$, HPLC grade nitric acid and deionized water.

Standards Preparation

2.20g of pure nickel solution was dissolved in 50ml of 1M HNO_3 solution in a 1L volumetric flask. After dissolution, the flask was made up to the mark with deionised water to form 1000ppm. (Mendham, 2002). Further dilutions were undergone and the following working standards were obtained (0.001, 0.002, 0.003, 0.004, 0.005, 0.006ppm up to 1ppm). A similar procedure to that described above for the preparation of standard solution of nickel was followed to prepare 0.001ppm – 1ppm of copper, zinc and lead solution respectively using their appropriate weights.

Procedure for Wet Digestion

1.00g of the muscle tissues of the fish sample was weighed in each case and transferred to a 100cm^3 beaker. 5 cm^3 of nitric acid was added to the content and boiled for two minutes. 10 cm^3 of deionized water was added to the mixture and was made up to 100 cm^3 with the same deionized water. Blank experiment was carried out with deionized water instead of the sample involving all the reagent and procedures used for the actual digestion. Solutions were analyzed using atomic absorption spectrophometer.

IV. STATISTICAL ANALYSIS

Data were analyzed using application soft wares such as Microsoft excel and Microsoft word version 2007. Data were presented as mean and standard deviation.

V. RESULTS

As presented in table 1, the mean weight of tilapia fish samples used was 94.700$\pm$11.455 g and the ranges of their variable weights were 86.60-102.80 g. Also the mean length used for the fishes was 18.333$\pm$0.153 cm with variable lengths ranges between 18.2 -18.5 cm.

Table 1. Weights and lengths in triplicate of the Tilapia zilli before drying

Parameter	Pond	No. of fish	Weight (g)	Length (cm)
	Gwale	3		
Range			86.60-102.80	18.2-18.5
Mean and SD			94.700$\pm$11.455	18.333$\pm$0.153

The mean concentrations of the four heavy metals analyzed in muscle tissues of tilapia zilli were presented in table 2 as shown below.

Table 2. Mean, standard deviation, 95% confidence interval, LOD, LOQ and experimental error of triplicate analysis of muscle tissues of Tilapia zilli.

Heavy Metals (ppm)				
Replicates	**Nickel (Ni)**	**Copper (Cu)**	**Zinc (Zn)**	**Lead (Pb)**
1	0.01	0.0600	0.0100	0.0070
2	0.0120	0.0640	0.0090	0.0075
3	0.0140	0.0670	0.0100	0.0080
Mean	0.0120	0.0637	0.0097	0.0075
Std.Dev. (S)	0.0020	0.0035	0.0006	0.0005
CV (%)	16.67	5.49	6.19	6.67
LOD	0.006	0.011	0.002	0.002
LOQ	0.02	0.035	0.006	0.006
95% CI	0.0120 $\pm$ 0.005	0.0035 $\pm$0.009	0.0006 $\pm$0.001	0.0005 $\pm$0.001

Error	0.008	-0.044	0.010	0.012

Key: SD = Standard Deviation; CV = Coefficient of Variation; LOD = Limit of Detection; LOQ = Limit of Quantitation; CI = Confidence Interval.

From table 2, the mean concentration of nickel, copper, zinc and lead were 0.0120$\pm$0.002 ppm, 0.0637$\pm$ 0.0035 ppm, 0.0097$\pm$0.0006 ppm and 0.0075$\pm$0.0005 ppm respectively where as the ranges of their concentrations were 0.01-0.014 ppm, 0.06-0.067 ppm, 0.009-0.01 ppm and 0.007-0.008 ppm respectively. Figure 1, also illustrated the concentrations of nickel, copper, zinc and lead in ppm for each run and gives a systematic distributions of the individual concentrations in all the heavy metals analyzed.

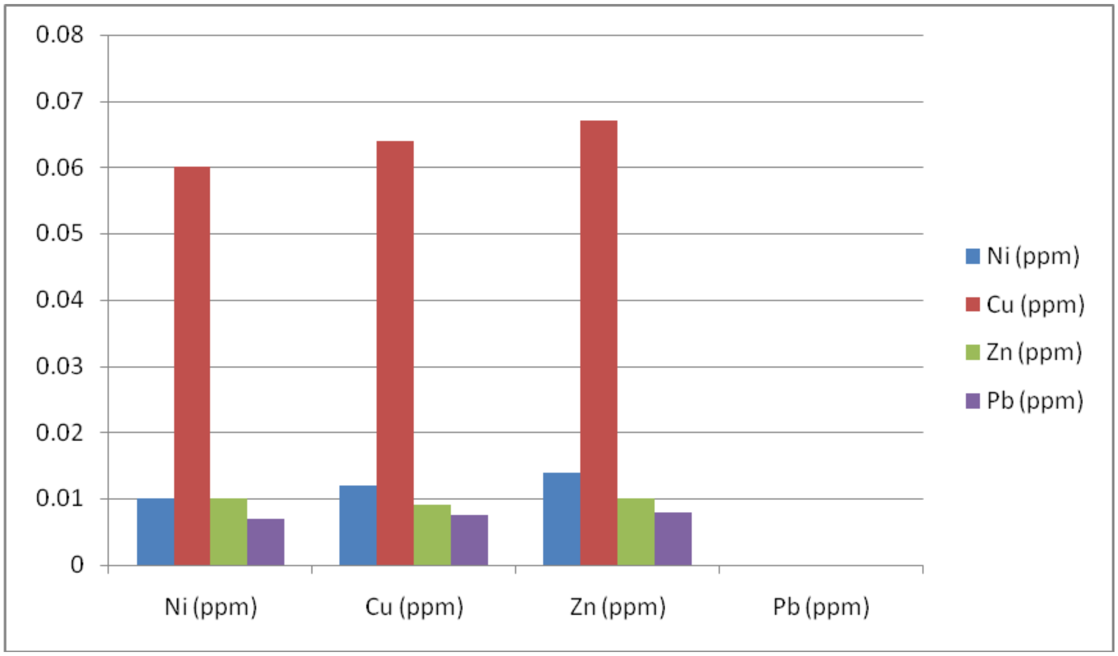

Figure 1. Graphical representation of the mean concentrations of the heavy metals metals in Tilapia zilli

Figure 2 also highlighted a graphical distribution of the heavy metals concentrations in the tilapia fish species and the extent of the absorption of all the heavy metals analyzed by the tilapia zilli may all be seen in the figure.

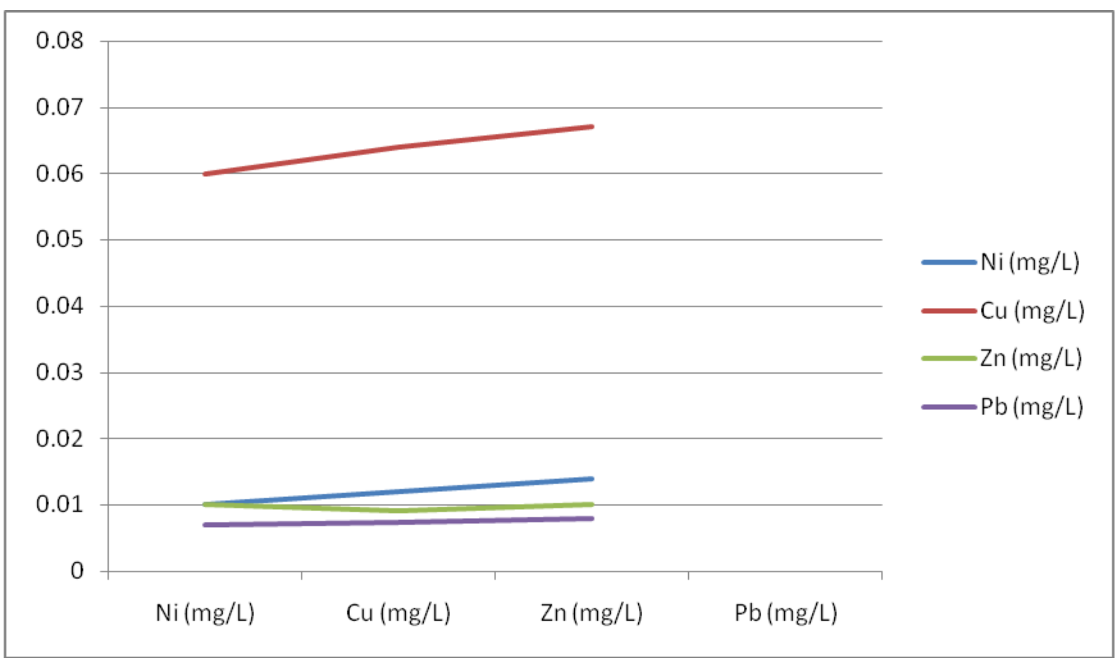

Figure 2: A line graph showing the mean concentrations of the heavy metals in Tilapia zilli

VI. Discussion

In this study, the concentrations of nickel, copper, zinc and lead were assayed in muscle tissues of three tilapia zilli specie in Gwale Pond of Kano State, Nigeria, in order to investigate the extent of heavy metals contamination of this water and also to draw attention to the general public on the dangers that may evolve as a result of frequent utilization of the fish species evolved in this pond having highlighted the toxicological and health risk effects which might cause a great risk to human wellbeing. The measurement of each metal was done in triplicate for effective tabulation.

The concentration of nickel ranged from 0.01-0.014 ppm, with a mean of 0.0120 ppm. This shows that, the mean concentration of nickel was below [14] limit of 0.4 ppm. The mean concentration of copper was 0.0637 ppm and it ranges were 0.060-0.067 ppm. This shows that, the mean concentration of copper was below[14] limit of 1.5 ppm. The ranges of concentration of zinc were 0.0006-0.01 ppm where as its mean concentration was 0.0097 ppm. This shows that the mean concentration of zinc in tilapia zilli was far below the toxic level of 15 ppm [14]. The mean concentration of lead was calculated to be 0.0075 ppm where as its ranges were 0.007-0.008 ppm. This shows that the mean concentration of lead was less than the toxic limit of 1.5 ppm [14]. This method is validated using triplicate analysis and recovery experiment and statistical analysis as presented in table 1. From table 2, the limit of detections (LODs) of nickel, copper, zinc and lead were found to be 0.006, 0.011, 0.002 and 0.002 whereas the limit of quantitations (LOQs) were recorded as 0.02, 0.035, 0.006 and 0.006 respectively. Also the 95% confidence intervals for nickel, copper, zinc and lead were calculated to be 0.0120 $\pm$ 0.005, 0.0035 $\pm$0.009, 0.0006 $\pm$0.001 and 0.0005 $\pm$0.001 respectively. The experimental error generated was 0.008 for nickel, -0.044 for copper, 0.010 for zinc and 0.012 for lead.

In comparison with other studies, the mean concentration of zinc and lead were far below those reported from Asa river of Ilorin, Kwara State, Nigeria [15]. Similar work on similar fish species has been reported by (Ibrahim, S. and Kassim, J., 2012), but with lower mean concentrations of zinc metal (4.337 $\pm$1.732, 3.447$\pm$1.296 mg/kg) and lead (0.055$\pm$ 0.023, 0.038$\pm$0.037 mg/kg) both in Aisami Sewage Pond of Gwale and Dala Pond of Kano State, Nigeria. Figure 1 and 2, represents the graphical mean concentrations of the heavy heavy metals in the samples analyzed.

Consequently, copper metal has the highest mean concentration from the results where as lead metal has the lowest mean concentration as shown in figure 1 and 2.

VII. Conclusions

The present study showed that there is a presence of contamination of nickel, copper, zinc and lead in Gwale Pond. The mean concentrations of nickel, copper, zinc and lead were 0.0120$\pm$0.002 ppm, 0.0637$\pm$ 0.0035 ppm, 0.0097$\pm$0.0006 ppm and 0.0075$\pm$0.0005 ppm respectively. However the mean concentrations of the heavy metals analyzed fell below the minimal risk level of 0.4 ppm nickel, 1.5 ppm copper, 15 ppm zinc and 1.5 ppm lead [14]. In the same vein, the 95% confidence intervals for nickel, copper, zinc and lead were calculated to be 0.0120 $\pm$ 0.005, 0.0035 $\pm$0.009, 0.0006 $\pm$0.001 and 0.0005 $\pm$0.001 respectively. The order of increasing mean concentrations of the four metals analyzed is given as; Pb<Zn<Ni<Cu.

Further study should also be carried out for other metals using the same and different techniques in order to have a remarkable database for heavy metals determination in both food stuffs and environmental products.

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